Cell Biology

EXPRESSION OF WILD-TYPE AND MUTANT α-SYNUCLEIN IN A YEAST MODEL FOR PARKINSON'S DISEASE

Brandon E. Johnson, Shubhik K. DebBurman*
Lake Forest College: 555 N. Sheridan Rd; Lake Forest, IL 60045. johnsbe@lfc.edu.

Parkinson's disease (PD) is a fatal, incurable human neurodegenerative disease that affects approximately 4 million worldwide. α-Synuclein is associated with both familial and sporadic PD. α-Synuclein misfolds and aggregates within dopaminergic neurons that selectively die in PD, but molecular mechanisms that cause protein misfolding and link it to cell death are still not fully understood. α-Synuclein overexpression has recently been shown to cause PD-like pathology and symptoms in fruit flies and mice. Till date, no yeast model for PD has been reported. Given the present ability to genetically manipulate yeast, we support the hypothesis that such a model will facilitate understanding of processes governing and reversing synuclein misfolding. The aims of this study are four-fold: to transform and express α-synuclein into yeast, determine intracellular localization, identify partner proteins that bind to it, and characterize its tertiary structures. Wild-type and three α-synuclein mutant forms were subcloned into a galactose inducible expression vector, and transformed into yeast. Western blot analysis demonstrated that expression of both wild-type and mutant α-synuclein were induced to similar levels, while a yeast housekeeping protein RSP5 was expressed independent of the galactose induction. Current experiments in protein purification, immunoflourescence microscopy, co-immunoprecipitation and protease digestion are being performed to comparatively examine the localization and molecular nature of wild type and mutant α-synuclein. We hope that this yeast model will ultimately allow us to genetically dissect factors that regulate synuclein misfolding.